

In Claim 43, claim line 3, delete "1 or 2", and substitute therefor --I or II--.

REMARKS

Claims 1-27 and 35-43 are pending in the application. Claims 28-34 have been withdrawn pursuant to a restriction requirement. Claims 7, 17, 20-22, 24, 26, 27, 42, and 43 have been amended.

Support for the amendments are as follows. The reference to the parent application in the Specification has been updated to include the patent number of the parent application.

In Claims 7, 17, 20-22, 24, 26, and 27, the periods following each of the steps of the claims have been deleted and replaced by parentheses as requested by the Examiner. In Claims 22-24, the steps have been denoted with the letters i, ii, etc as requested by the Examiner. Claim 22 has been amended to replace "an HLA-locus specific primer pair" with --said primer pair-- to clarify the antecedent basis in Claim 21.

In Claim 27, the clearly omitted step of digesting the amplified DNA sequence from the child's mother produced in step a and compared in step c has been inserted. The claim was part of the original disclosure of the application. In addition, it is clear that the patterns produced by the maternal and child's DNA are compared with that of the putative father to determine if the putative father has the obligate paternal allele. (See the Specification at page 69, lines 23-34 and Example 2, particularly page 83, lines 10-16.)

In Claims 42 and 43, the arabic numerals have been replaced by the roman numerals as requested by the Examiner. In addition, in Claim 42, IVS IV has been deleted.

Therefore, no new matter is added by any of the amendments.

Claim to Priority

The Examiner stated that a specific reference to the parent application must be made in the specification to claim priority under §120. The Specification was amended to contain such a reference in the filing papers. (See page 2 of the Division-Continuation Program Application Transmittal Form.) The reference has now been updated to include the patent number, which patent had not issued when the application was filed.

Restriction Requirement

Applicant confirms the election of claim group I with traverse. Reconsideration of the restriction requirement is requested. The claim groups are as follows:

- I Claims 1-27 and 35-43, drawn to methods to detect DNA polymorphism (Class 435, subclass 6)
- II Claims 28-34, drawn primers, amplified DNA and a kit comprised thereof (Class 536, subclasses 23.1, 24.3 and 24.33)

The two criteria which must be met for a proper restriction requirement between patentably distinct inventions are (1) that the inventions must be independent or distinct as claimed; and (2) that there must be a serious burden on the examiner if restriction is not required (MPEP 803). Although the inventions of the claim groups are independent and distinct from each other, examining all of the claims in one application does not place a serious burden on the examiner.

The two claim groups are closely related. Specifically, the primers, amplified sequences and kits of Group II are used in the methods of Group I. Joining the claims in one group means searching three one additional subclasses for a total of 4 subclasses. Because the subject matter of the claims of Groups I and II are closely related, the claim groups can be efficiently examined together. Since examination of all of the

claims does not place a serious burden on the examiner, those claim groups are properly joined in one application. Reconsideration and withdrawal of the restriction requirement is respectfully requested.

Information Disclosure Statement

A 1449 which lists the titles of the articles cited in the preliminary amendment together with the article submitted herewith accompanies this amendment. Citation of the articles is respectfully requested.

Informalities

The disclosure was objected to for informalities. Each of the informalities noted by the Examiner has been corrected as requested.

Rejection under 35 U.S.C. §112, Second Paragraph

The rejection of Claims 22 under 35 U.S.C. §112, second paragraph, for indefiniteness is believed to have been overcome by the amendment to the claim which makes it clear that the primer pair of Claim 22 is the same as that of Claim 21.

Rejections under 35 U.S.C. §112, First Paragraph

The objection to the Specification and rejection of Claims 1-20, 27, and 35-43 under 35 U.S.C. §112, first paragraph, is in part overcome by amendment and in part traversed, as explained in detail below.

Claim 27

The rejection to Claim 27 as not enabled for failing to recite that the mother's amplified DNA has been digested has been overcome by amending the claim to recite that the mother's amplified DNA has been digested. Withdrawal of the rejection is respectfully requested.

Claims 1-6

The Examiner stated that Claims 1-6 are drawn to a detection method yet fail to recite a detection step and are therefore not enabled. That rejection is respectfully traversed. First, the method recites that the amplification is performed "to produce an amplified DNA sequence characteristic of said allele." Therefore, it is clear that the amplification detects the allele since the resultant amplified sequence is characteristic of the allele.

Second, the law clearly indicates that the enablement requirement means that the application enables one of ordinary skill in the art to make and use the invention. The claims do not need to teach one how to perform the method.

In In re Ranier et al., 134 U.S.P.Q. 343 (C.C.P.A. 1962), claims were rejected as unduly broad because the minimum energy level was not stated in the claims. The Court stated that the Board of Appeals found that reliance on the element stated in the claim would involve extensive experimentation for ascertaining the practical limits of operation. The Court stated:

"It appears to us that the board is here confusing the requirements for claims with the function of specification. One does not look to the claims to find out how to *practice* inventions they define, but to specification. Here the "practical limits of operation" are set forth in the specification so as to eliminate any need for "extensive experimentation."" (emphasis in original; 134 U.S.P.Q. at 346)

Therefore, the claims need to be enabled by the teachings of the specification. The claims do not need to recite all the necessary steps to enable one to practice the claimed method. Since the Examiner based the rejection on the lack of a step reciting detection, not on the failure of the specification to teach one of skill how to detect the allele, the claims are enabled and comply with the statutory requirements. Withdrawal of the rejection is respectfully requested.

Claims 35 and 36

Claims 35 and 36 were rejected for lack of support in reciting that the genetic locus has at least 4 (Claim 35) and at least 8 alleles (Claim 36). The Examiner states that the support for the number of alleles for the locus was pointed out in relation to HLA loci, not any locus and that Claim 1, on which Claims 35 and 36 depend relates to analyzing general loci, not analyzing HLA loci.

As is clear to the Examiner, the HLA loci are a particular group of loci which are encompassed within the claim to general loci. Specifically, like Claim 1, Claim 7 relates to general loci, not just to HLA loci. Claim 12, which depends on the analysis method for general loci recited in Claim 7 recites that the genetic locus is a major histocompatibility locus. The major histocompatibility loci include the HLA loci and similar loci of other species. Therefore, it is clear even within the structure of the claims that the HLA loci are examples of the general loci that can be analyzed by the claimed method.

The Examiner also recognizes that the Specification explicitly contains the support for the HLA alleles, a particular subset of the general loci to be analyzed by the method. Therefore, it is clear that for some loci encompassed by the terms of Claim 1, the HLA loci, the loci can have 4 or 8 alleles. No further support for the claims is required. Withdrawal of the rejection is requested.

Claims 37-43

The Examiner stated that Claims 37-43 "recite a method to analyze by identifying "sequence polymorphism" characteristic of the alleles." (page 5) The Examiner states that the support for term "sequence polymorphism" was not pointed out and that the support for the term appears to be in Dr. Gresshoff's declaration. The Examiner states that the failure

of the application to support the use of sequence polymorphisms introduces new matter. The rejection is respectfully traversed.

The application as filed supports claiming the use of sequence polymorphism located in non-coding regions to determine alleles and haplotypes. The application states:

"The amplified DNA sequence is analyzed to detect the presence of a genetic variation in the amplified DNA sequence such as a change in the length of the sequence, gain or loss of a restriction site or substitution of a nucleotide. The variation is characteristic of the allele to be detected.

The present invention is based on the finding that intron sequences contain genetic variations that are characteristic of adjacent and remote alleles on the same chromosome." (Specification, page 7, lines 26-36)

"The amplified DNA sequence is analyzed to detect the presence of a genetic variation such as a change in the length of the sequence, gain or loss of a restriction site, or substitution of a nucleotide." (Specification, page 8, line 35 through page 9, line 3)

Therefore, it is clear that the application as filed supported the use of genetic variations in non-coding region sequences. The genetic variations include all possible variations such as insertion of one or more nucleotides, deletion of one or more nucleotides or substitution of one or more nucleotides. Such genetic variations in a nucleotide sequence are also referred to as polymorphisms in the sequence. Therefore, the use of sequence polymorphisms is supported by the application. If the Examiner wants the term changed to the synonymous "genetic variation", please inform Applicant's Attorney. Withdrawal of the rejection is respectfully requested.

Claim 38

The Examiner states that the recitation of "not more than about one kilobase in length" in Claim 38 is not supported by the Specification. If the Examiner wants the claim amended to state that the sequence is "about one kilobase in length" or is "about 1000 nt in length", Applicants will amend the claim. It

is clear that 1000 nt is one kilobase so the basis for the rejection is not understood. Clarification is requested.

Claims 42 and 43

The Examiner rejected Claims 42 and 43 for reciting that IVS I, II, III, or IV (for Class I) (now amended to recite IVS I, II, or III) and IVS I or II (for Class II) for introducing new matter. Claim 42 has been amended, and the rejection is respectfully traversed.

As noted by the Examiner, the Specification indicates that the second and third exons are the variable exons for Class I and the second exon is the variable exon for Class II. At page 43, lines 16-21, the Specification states that preferably the amplified DNA sequence contains all exons that encode allelic variability together with at least a portion of one of the adjacent intron sequences. Therefore, the Specification clearly supports that the introns adjacent to (on one side or the other of) the variable exons are used. For Class I, the introns adjacent to the variable exons are IVS 1 and 2 (adjacent to exon 2; IVS I is between exon 1 and exon 2 and IVS II is between exon 2 and exon 3), and IVS 2 and 3 (adjacent to exon 3). For Class II the introns adjacent to the exon 2 are IVS 1 and 2. Therefore, no new matter is introduced. Removal of the rejection is respectfully traversed.

Claims 1-16 and 37-41

The rejection of Claims 1-16 and 37-41 for lack of enablement is respectfully traversed. As stated by the Examiner, the Examiners who interviewed the parent application rejected the broader analysis claims since the evidence in the application and presented at the interviews supported HLA, but did not convince the Examiners that the method was operative for loci of eukaryotic organisms generally. Applicant intended

to submit either preliminary data related to CF or data related to other loci by way of a Declaration of an Expert.

The Examiner stated:

"the only submission regarding the general application of the method is from Dr. Gresshoff; the Declaration however is not acceptable because it discloses that sequence polymorphism was found in the intergenic region, not in the intron or intron/exon regions as originally disclosed." (page 6)

As stated by the Examiner, Applicant proposed submitting data regarding the CF locus. However, the Examiners in the parent application were uncertain whether such additional data by itself would convince the Examiners that the method was generally applicable. Therefore, the Examiners indicated that additional data together with the Declaration of an expert who carefully explained why the additional data demonstrated the general applicability of the method should be submitted. Therefore, the Examiners did not consider the submission of data related to any particular locus to be critical, but wanted the data to be convincing to an expert and to clearly understand the reasons why the expert was convinced. After analyzing the reasoning by the expert, the Examiners would decide if the Declaration was sufficient to overcome the rejection.

The Declaration of Dr. Gresshoff is based on data that convinced Dr. Gresshoff that the present method was generally applicable to eukaryotic loci. The data related to an intergenic region near the soybean supernodulation (NTS) locus, a conserved locus in a plant. The Examiner stated that the Declaration was based on intergenic sequences and that the application relates to intron sequences. However, the application relates to non-coding region sequences. As described in the specification, the non-coding regions useful in the method include intergenic regions. (See the Specification at page 10, line 29 through page 11, line 4, particularly page 10, lines 32-36 which state that intergenic regions are included within the term "intron".) Therefore,

Applicants have presented additional data, which data convinced an expert that the method was generally applicable.

Dr. Gresshoff presented reasons explaining why the soybean data was convincing in addition to the HLA data presented by Dr. Simons. Dr. Gresshoff stated that he did not perform these studies to determine whether Malcolm Simons' work applied to plants. He simply was working in that intergenic region of the soybean genome and found the pattern of variation. Since he has no reason to believe the region is anything other than typical of intergenic regions of the soybean genome or of the genomes of other plants, he believes that "there is no reason to expect that other regions of the soybean genome or any other plant genome would be different."

In addition, Dr. Gresshoff stated that although the NTS gene is a conserved gene, "the same correlation of non-coding region polymorphisms with coding region polymorphisms which is present in the HLA genes is also present in the soybean NTS gene." Dr. Gresshoff stated that when he only was aware of the HLA data, he was concerned that the phenomenon could be related to members of the immunoglobulin super-gene family or to gene families with high coding region variability. Any such concerns were clearly eliminated by observing the same phenomenon in a conserved gene.

Dr. Gresshoff also stated that in addition to demonstrations of this non-coding region micro-heterogeneity in both highly polymorphic and conserved genes, the data were obtained in humans and in soybeans. "Clearly, this indicates that the phenomenon is not limited to humans or even animals." Dr. Gresshoff believes that the presence of non-coding region polymorphisms that are indicative of coding region polymorphisms (alleles) in such phylogenetically distant species indicates that the phenomenon is present in eukaryotic genomes generally.

In addition, Applicant's Attorney notes that the sequence variations found by Dr. Gresshoff were in an intergenic region, rather than in an intervening sequence. Therefore, the useful non-coding region sequences are not limited to those associated with a genetic locus, such as the introns or flanking regions of the gene.

Therefore, Dr. Gresshoff described the data regarding a conserved locus in the soybean. The data convinced Dr. Gresshoff that the correlation of non-coding region polymorphisms with coding region polymorphisms which is present in the HLA genes and the soybean NTS gene is present in eukaryotic genomes generally. Dr. Gresshoff also presented reasons why the data was convincing and demonstrated that the correlation is present in eukaryotes. Therefore, the Declaration of Dr. Gresshoff meets the criteria of the Examiners.

As stated above, Applicants submitted the Declaration of Dr. Gresshoff under 37 C.F.R. §1.132 to overcome a rejection based on the Examiners' concerns that the method had not been shown to be effective in analyzing genetic loci, generally. The criteria for evaluating declarations traversing rejections are set forth in M.P.E.P. 716. The criteria are (1) the declaration is timely filed; (2) the declaration must set forth facts, not merely conclusions and the facts must be pertinent to the rejection; (3) the declaration should be scrutinized closely and the facts presented weighed with care, particularly if the declarant is interested in the outcome of the case.

In response to the 35 U.S.C. §112 rejection, the Declaration was submitted with a preliminary amendment. Therefore, the declaration was timely. In his declaration, Dr. Gresshoff described data he developed in studying the soybean NTS locus. Dr. Gresshoff described that in his work, he also found that there were sequence polymorphisms in the intergenic region of the locus which indicated the allele of

the gene. Therefore, Dr. Gresshoff provided facts, not merely conclusions.

The facts presented by Dr. Gresshoff are pertinent to the rejection because the facts relate to whether non-coding region polymorphisms in non-HLA genes could be used to identify alleles. The region described by Dr. Gresshoff is an intergenic region. As described in the specification, the non-coding regions useful in the method include intergenic regions. (See the Specification at page 10, line 29 through page 11, line 4, particularly page 10, lines 32-36 which states that intergenic regions are included within the term "intron".) Therefore, the facts are pertinent to the rejection.

Finally, Dr. Gresshoff is neither a coinventor, nor an employee of the assignee of the application. He is a colleague of Dr. Simons who is interested in Dr. Simons' work. Therefore, the statements made by Dr. Gresshoff are not made to further any interests of Dr. Gresshoff.

Since the Declaration complies with all the requirements of M.P.E.P. 716, the Declaration must be considered by the Examiner. Since the Examiner misunderstood the relevance of the facts recited in the Declaration, as indicated by the statement that the application did not relate to intergenic region sequences, reconsideration of the Declaration and withdrawal of the rejection is respectfully requested.

The Examiner also stated that "a rejection on the newly introduced matter (the Declaration) is also being made." The basis for this rejection is not understood. The Declaration responds to a rejection and is in compliance with M.P.E.P. 716. Applicants have not attempted to enter information from the declaration into either the specification or the claims. M.P.E.P. 608.04 relates to entering new matter into the specification, the claims, or the drawings.

Under M.P.E.P. 608.04, the Examiner is required to object to the entry of new matter into the specification and require

cancellation of any such new matter and to reject claims affected by new matter. That a declaration under 37 C.F.R. §1.132 contains data not present in the application as filed does not provide a basis for a rejection based on new matter. Since submitting a declaration neither amends the claims, the specification nor the drawings, no new matter has been entered by submission of additional information by way of a declaration.

If the rejection based on new matter is repeated, clarification of the basis for the rejection is respectfully requested. Since Applicant was unable to address this rejection, Applicant requests that any office action which repeats the rejection be a non-final office action.

Also accompanying this Amendment is a Declaration by Leroy Hood. As is well known and evidenced by his Curriculum Vitae, Dr. Hood is an expert in genetics.

In his Declaration, Dr. Hood describes that he sequenced a 100 kilobase region of the Alpha Delta T-cell receptor gene in both mouse and man. Ninety-five percent of the sequenced region was non-coding. Yet Dr. Hood found the homology between the human and mouse sequences was approximately 70%. Dr. Hood states that 70% is approximately the percentage of homology that many coding regions between mouse and man exhibit. Dr. Hood found this observation "a great surprise" because the T-cell receptor genes are a paradigm of the diversity genes. He stated that he therefore did not expect to find a high level of homology between the mouse and human sequences.

Dr. Hood also reviewed data provided by Dr. Simons related to related to HLA genes. Dr. Hood states that the "data provided by Malcolm Simons related to his discovery that one could use relatively short regions of non-coding sequences, on the order of one to two kilobasepairs, to define the corresponding coding region allele." Dr. Hood states that Malcolm Simons' "data demonstrated that relatively short non-

coding region sequences contained informative polymorphisms which can be used as the basis of an HLA typing system." Dr. Hood also saw the Declaration by Peter Gresshoff submitted with the Preliminary Amendment which stated that Dr. Gresshoff has observed similar informative polymorphisms in an intergenic region near the nitrogen tolerant symbiosis (NTS) gene in soybeans which polymorphisms were indicative of the co-cultivar.

Dr. Hood stated that Dr. Simons' data demonstrates that the percentage of homology of non-coding regions and of coding regions in different alleles of the HLA loci is approximately the same. Dr. Hood further stated that his data on the T-cell receptor gene "demonstrated a similar phenomenon between genes of species which diverged approximately seventy to eighty million years ago." Dr. Hood found the phenomenon in the T-cell receptor gene particularly striking because the gene is a paradigm of the diversity genes.

Dr. Hood concluded:

"The HLA data and the NTS data indicate the presence of informative polymorphisms in non-coding in these vastly different types of genes from species that diverged tens of millions of years ago. In addition, the type of homology I found in sequencing non-coding regions of mouse and man in the Alpha Delta T-cell receptor is consistent with these findings. Therefore, I believe that informative polymorphisms which are indicative of linked alleles and haplotypes are present throughout the eukaryotic genome."

Like the Declaration by Dr. Gresshoff, Dr. Hood's Declaration complies with the three criteria for evaluating declarations traversing rejections as set forth in M.P.E.P. 716. First, the declaration is timely filed in response to the first Office Action. The declaration sets forth facts pertinent to the rejection, not merely conclusions. Dr. Hood described his data regarding a 100 kilobase region of the Alpha Delta T-cell receptor gene in both mouse and man. The data is pertinent in that 95% of the sequenced region was non-coding sequences. Finally, Dr. Hood was contacted by Dr. Simons who showed Dr. Hood his data. When Dr. Hood commented on Dr.

Simons' data and compared Dr. Simons' data to data Dr. Hood had generated, Dr. Simons asked Dr. Hood if he would be willing to prepare a declaration in support of the patent application. Like Dr. Gresshoff, the statements made by Dr. Hood are not made to further any interests of Dr. Hood.

Dr. Hood concluded that the data indicated that informative polymorphisms which are indicative of linked alleles and haplotypes are present throughout the eukaryotic genome. Dr. Gresshoff also concluded that the presence of non-coding region polymorphisms that are indicative of coding region polymorphisms (alleles) indicates that the phenomenon is present in eukaryotic genomes generally. The present method is based on the use of informative polymorphisms in non-coding regions. There is now clear evidence of record that the data indicates that such informative polymorphisms are present throughout the eukaryotic genome. The evidence clearly demonstrates that the present method is not limited to HLA genes but rather is applicable to eukaryotes generally.

Also accompanying this Amendment are Abraham et al. [*Tissue Antigens* 39:117-121 (1992)] and Reiß et al. [*Immunogenetics* 32:110-116 (1990)]. Those articles are cited as references AR and AS, respectively, on Sheet 7 of 7 of the accompanying Form PTO-1449. The Abraham article describes two intergenic regions, designated CL1 and CL2, which are located between HLA-B (in the HLA Class I region) and TNF (in the central MHC region). The authors state that polymorphisms in the regions are indicative of HLA-B and HLA-C haplotypes and can be used to determine the haplotypes.

The Reiß article describes sequences in the second intron of the HLA-DRB gene which distinguish DRB allele groups. In particular, the authors state that the repeats in the second intron can distinguish subgroups of identical DRB1 alleles (i.e. haplotypes). In addition, the repeats distinguish the DRw52 supergroup from the DRB1*0101, DRB4*0101 and DRB5*0101

and from those of pseudogenes. Therefore, other scientists have also found polymorphisms in intergenic regions and in introns that correlate to HLA alleles and haplotypes.

The opinions of experts in the field evidences that the data presented to the Examiner demonstrates that informative polymorphisms which are indicative of linked alleles and haplotypes are present throughout the eukaryotic genome and are not limited to HLA genes. In addition, the newly cited articles evidence that other investigators have also identified informative polymorphisms which are indicative of HLA alleles and haplotypes, confirming Dr. Simons' discovery.

In light of the clear evidence of record that the claimed method is reproducible and applies to genetic loci generally, and not just to HLA loci, withdrawal of the rejection is respectfully requested.

Claims 17-20

The rejection of Claims 17-20 under 35 U.S.C. §112, first paragraph, as overbroad is respectfully traversed. The Examiner states that the disclosure is enabling only for claims limited to primer sites which span a non-coding region where the primer pair defines a sequence in linkage with the HLA locus. The Examiner states:

"The claims as recited are broad in scope (an allele of a HLA locus could be found in both the coding and the non-coding region), which is not supported by the original disclosure: the polymorphism is found in the introns, not in any region of the HLA locus."
(page 6)

Applicant's Attorney would like to explain the technical background of the invention so that the Examiner can better understand the scope of the disclosure of the application.

As used in the art in reference to HLA alleles (and defined in the Specification at page 10, lines 5-8), the term "allele" refers to variations in the coding region of a gene. That is, each coding region variant of a gene is an allele of the gene. Therefore, as defined in the application, alleles

necessarily refer to coding regions of the gene. The Examiner's statement that alleles of the HLA locus can be found in both the coding and non-coding region of an HLA locus is incorrect. The term "alleles" refers to polymorphisms in the coding regions.

Second, polymorphisms are found in both the coding regions and the non-coding regions of the HLA loci. In particular, the number of alleles of the various HLA genes indicate the number of different patterns of polymorphisms in the coding region of the HLA locus. For example, the DQA locus has at least eight alleles, meaning at least eight different patterns of polymorphism are present in the coding region of the gene. As discussed earlier, the predominance of the variation is in Exon 2 (DQA is in HLA Class II). Because the majority of the polymorphisms are in the about 250 nt of Exon 2 and the polymorphisms are non-unique (the same substitution appears in more than one allele), analysis of the coding region has not provided unambiguous results. For example, a probe which is sufficiently large to bind to a region of the exon encompassing a plurality of the polymorphisms may bind to the coding region of more than one allele since there are so few mismatches.

The basis of Applicant's invention is that variations (polymorphisms) in the non-coding regions are also indicative of the coding region allele. However, since there are so many more non-coding region nucleotides, Applicant was able to identify polymorphisms indicative of a single allele of the locus. That is, Applicant was able to identify unique polymorphisms or unique polymorphic patterns for each allele. Prior to Applicant's invention, the non-coding regions were thought to accumulate meaningless polymorphisms and were referred to as junk.

Turning now to the rejection, the HLA alleles are coding region polymorphisms in the variable exon(s) of the HLA genes. As stated in the Specification at page 15, lines 3-12, for some

HLA loci, such as DPA, the number of alleles is sufficiently small to permit amplification of the exon region only. Therefore, there is clear support that the HLA analysis method does not necessarily require use of intron sequences. Withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. §103

The rejection of Claims 17-20 under 35 U.S.C. §103 over Erlich (U.S. Patent No. 4,582,788) in view of Saiki (U.S. Patent No. 4,683,194) is respectfully traversed. The Examiner states:

"Erlich discloses a RFLP HLA typing method (claim 1) and a nucleotide sequence of an HLA locus (clone 18C7 sequence in columns 9 and 10). Erlich fails to teach amplifying the locus instead of using whole genomic DNA for digestion. Saiki et al. disclose an amplification/RFLP method which comprises amplification of target DNA and digestion thereof with restriction endonuclease (Summary of the Invention).

It would have been prima facie obvious to one of ordinary skill in the art to amplify a HLA locus before digestion for RFLP analysis because the HLA sequence is known (Erlich) and the PCR/RFLP method has been disclosed (Saiki et al.). An ordinary skill in the art would have known to make the invention because gene amplification by PCR for DNA analysis (RFLP analysis) is much more sensitive and requires less starting amount of DNA than the conventional genomic digestion method." (page 7)

Erlich teaches classical RFLP methods for analysis of HLA Class II loci. As stated by the Examiner, Erlich uses whole genomic DNA for digestion. Following digestion, Erlich uses a probe in a blot to identify fragments of the genome related to a selected HLA region. The pattern of lengths of the fragments are determined to identify the allele present in the sample.

In Example 6, the method was used to produce patterns from three individuals. As stated in the patent, each individual has a 986 bp fragment and a 6.8 kb fragment. In addition, every individual also had either a 9.2 or 13.0 kb fragment or both. The relative locations of the restriction sites for this analysis is illustrated in Figure 3. As can be seen in the figure, the sequence containing the sites spans 20.8 kb.

The Examiner stated that Saiki et al. "disclose an amplification/RFLP method which comprises amplification of target DNA and digestion thereof with restriction endonuclease (Summary of the Invention)." The references to use of amplification appears to be at column 6, lines 34-39 and in Example V, rather than in the Summary as stated in the Office Action. The Saiki patent relates to "a method for detecting the presence or absence of a specific restriction site in a specific nucleic acid sequence, through the use of specially designed probes." (column 3, lines 1-4) The probes are about 40 nucleotides in length, although probes which may be shorter or longer may be used. (column 3, lines 40-43) The probes are complementary to a region which spans the restriction site and are labeled at the end nearer the restriction site (column 3, lines 11-14).

The probes are hybridized to the sample DNA, then digested with the endonuclease. Any labeled cleaved oligomer is separated from labeled uncleaved oligomer and the presence or absence of labeled oligomer fragments is detected. Saiki states that the DNA can be amplified prior to the method and, in Example V, amplifies a region around the Dde restriction site in the β -globin gene to detect the sickle cell allele.

There is no motivation to combine the method of Erlich with that of Saiki. More specifically, Erlich determines alleles of HLA loci by producing characteristic fragment patterns. Those patterns span tens of kilobases. Although the Examiner states that Erlich provides the sequence of the HLA gene, the sequence is merely that of a portion of the coding region and does not show the sequence of the region of genomic DNA surrounding the restriction sites used to produce the characteristic patterns. Erlich does not provide sufficient information regarding the sequences surrounding the restriction sites to perform the Saiki method. Specifically, the sequence spanning the regions containing all the restrictions sites is

not provided so that the sequence to which the probes and the primers would bind are not known. In addition, the Erlich patterns are produced by a plurality of restriction sites spanning a region tens of kilobases in length. The PCR amplification method is not capable of amplifying sequences of such lengths. Therefore, at a technical level, one of skill could not practice the Saiki method on the Erlich sequences.

At a more fundamental level, one could not combine the Erlich and Saiki methods even if all the sequence information were known and one could amplify regions of 20 to 40 kilobases. Saiki amplifies around the region of a particular restriction site which is characteristic of the allele and detects the presence or absence of the site using a labeled probe which is cleaved when the site is present and remains intact when the site is absent. Erlich, on the other hand, uses genomic DNA with a single endonuclease to produce patterns which may differ between alleles of a locus and will distinguish some alleles of the locus. There is not a single restriction site, the presence or absence of which distinguishes the alleles. Instead a single endonuclease produces patterns having fragments of different lengths when used to digest extremely large regions of DNA.

The Erlich RFLP method is based on production of characteristic fragment patterns which span tens of kilobases. No one restriction site in a region of known sequence is informative. The Saiki method does not produce patterns. Instead, the method detects a single labeled oligonucleotide probe and determines whether the probe is intact or has been cleaved to determine whether the informative restriction site is present or absent. The Saiki method is dependent on detecting the presence or absence of a single informative restriction site in a region where the sequence surrounding the site is known. There is no reference which teaches or suggests that a single restriction site in a region of known or unknown

sequence is informative with HLA loci. In fact, Erlich teaches the opposite. Erlich teaches that combinations of restriction sites over large regions of DNA provide sufficient information to characterize some HLA alleles.

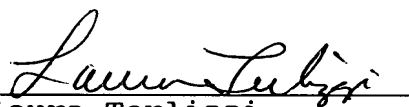
In addition, if one somehow could amplify the sequence spanning the region encompassing all the restriction sites used by Erlich (which is not possible because the sequence is too large), could design a probe which bound to the amplified sequence (which is not possible because the sequence information over that large region is unknown), and labeled the probe at one end as taught by Saiki, the probe would always be cleaved because a plurality of the sites are present in each region. Therefore, cleavage of the probe would happen irrespective of the allele present. The cleavage of the probe would not be informative.

Since neither of the references teaches how one would either identify a single informative restriction site for the HLA loci or how one could adapt the method of Saiki to detect a combination of restriction sites, there is no way to combine the methods of the references. Therefore, the combination of references does not make the present invention obvious.

In summary, the RFLP analysis described by Erlich is not amenable to combination with the PCR/RFLP method of Saiki because the Saiki method is designed to detect the presence or absence of a single restriction site of interest using a labeled probe that spans the restriction site. It is not the presence or absence of a restriction site in the genomic sequence that produces the Erlich RFLP patterns, it is the presence of various combinations of that site over tens of kilobases that produces the characteristic patterns. Therefore, there is no reason to combine the Erlich and Saiki references, and the combination does not make obvious the present method. Withdrawal of the rejection is respectfully requested.

All of the rejections having been overcome, it is believed that all of the claims are in condition for allowance. Early notice to that effect is respectfully requested. If a telephone conference would expedite prosecution of the above-identified application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,


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